Effect of phosphorus on arsenic transportation and accumulation in Pteris vittata L.

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論文内容要旨

Effect of phosphorus on arsenic transportation and accumulation in *Pteris vittata* L.

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Chapter 1: Introduction

Arsenic (As) is a highly toxic pollutant element that is ubiquitous in the environment and results from both natural and anthropogenic sources. Ingestion of inorganic As, the predominant form of As in drinking water, can increase the risk of cancers, such as skin, bladder and prostate.

Pteris vittata, the first identified As hyperaccumulator [1], has accumulated As in the fronds to concentrations as high as 22,630 mg/kg when grown in soil spiked with As at 1,500 mg/kg. *Pteris vittata* has also shown the ability to reduce the As concentration in contaminated water to a level that was within the World Health Organization (WHO) guidelines. The discovery of the As-hyperaccumulating capability of *P. vittata* not only opened the possibility for the phytoremediation of As-contaminated soil and groundwater, but also offered a unique model to study all aspects of plant–As interactions.

In this study the objectives were: ①, Characterization arsenate(As(V)) efflux from the roots of *P. vittata*. Specifically, we investigated the impacts of As concentration, As species and P levels on As(V) and arsenite(As(III)) efflux. ②, Determine the ability of phosphorus(P) accumulation in *P. vittata* and compare the P accumulation between the As-hyperaccumulator and non-hyperaccumulator. ③, Investigating the effect of P overaccumulation on As uptake, transport and accumulation in *P. vittata*. Such information helps to understand the mechanisms of As accumulation by *P.vittata*.

Chapter 2: Characterization of As efflux from the roots of As-hyperaccumulator *Pteris vittata* L.

Previous study showed that tomatoes (*Solanum lycopersicum*) took up As(V) and readily effluxed both As(III) and As(V)from their roots to the external medium [2]. It has been suggested that As efflux may be the general mechanism for As tolerance in plants. As(III) efflux was also observed during As(V) uptake in the hyperaccumulator *P. vittata* [3], but the characteristics of the As efflux from *P. vittata* remain unknown.

This study showed that both As(III) and As(V) were effluxed into the medium by *P. vittata* and the As(V) efflux was higher than As(III) efflux. The total As efflux was appreciably lower (6.88–21.1%) than for rice and tomatoes, where the majority (80–90%) of the As(V) that was absorbed during As(V) uptake was effluxed as As(III). These results suggest a limited As(V) efflux from As hyperaccumulator, *P. vittata*, as was suggested with As(III) efflux by Su et al. (2008), while both *P. vittata* and the non-hyperaccumulator, tomato, efflux As(V). The As hyperaccumulator *P. vittata* had a highly efficient As(III) xylem transport system compared with non-hyperaccumulating plants, but both the processivity of the efflux and the frond translocation were similar within 24 h, and low As(V) reduction capacity can reduce the As(III) efflux to the external medium (Fig. 1).

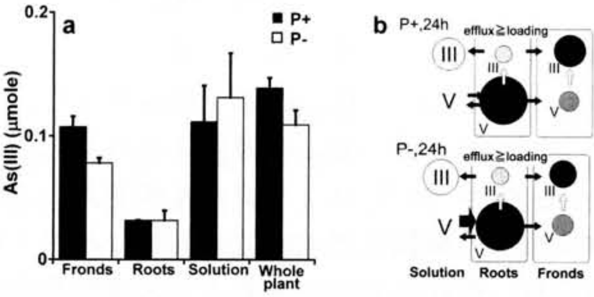

Fig. 1 a As(III) amount observed in each part of the plant following the 24-h As(V) uptake experiment by P+ *P. vittata* (closed bar) and P− *P. vittata* (open bar). b Schematic diagram of results shown in a where the area of the circle and the color shading in the circle represent the amount and concentration of the As, respectively. III As(III), V As(V). Data are shown as themean ± SE (n = 3)

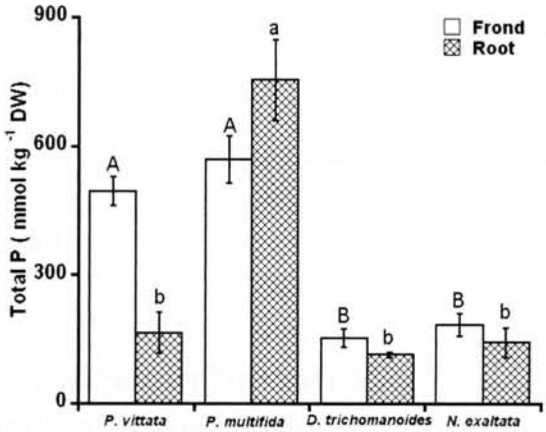

Fig. 2 Effects of high P supply (1,500 mg kg-1) on P concentrations in fronds and roots of *P. vittata*, *P. multifida* (As-hyperaccumulator) and *D. trichomanoides*, *N. exaltata* (As-non-hyperaccumulator). Data represent means of three replicates. The different lowercase / capital letters above the cross hatched / open columns indicate significant differences ($P < 0.05$) between them.

Chapter 3: Overaccumulation of phosphorus by As-hyperaccumulator *Pteris vittata* L.

Arsenic is toxic whereas phosphorus is essential for plants. In *P. vittata* they share the same transport pathway in root and same localization site (vacuole) in frond. P accumulation and As accumulation may have some relationship in *P. vittata*. To investigate the P accumulation ability in *P. vittata* need to applied high levels of P in growth media.

The results of present study showed that under high P cultivation (P 1,500 mg kg^{-1} P in soil or 3 mM in solution),

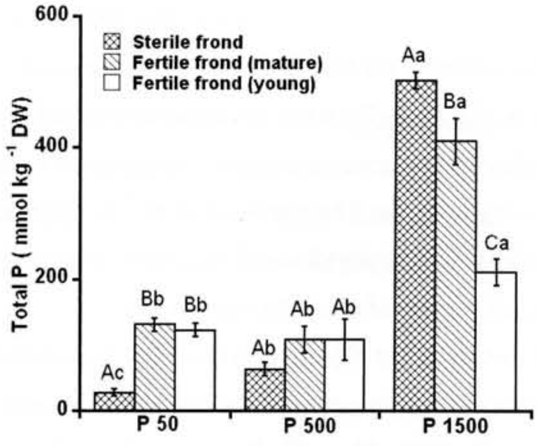

Fig. 3 Effects of different P supply on P concentrations in different fronds of *P. vittata*. Data represent means of three replicates. Different capital letters indicate that the different fronds under the same P treatment are significantly different; different lower case letters indicate that same fronds for different P treatment are significantly different ($P < 0.05$).

P transportation from root to frond was accelerated(Fig. 2). P was overaccumulate in fronds of *P. vittata* with suppression the P translocation from sterile frond to fertile frond and was associated with P phytotoxicity in fronds for the long term cultivation (Fig. 3). To our knowledge, this observation has not been reported before. P overaccumulation was only occurred in As-hyperaccumulator *P. multifida* and *P. vittata* but not in non-hyperaccumulator *D. trichomanoides* and *N. exaltata* suggested that P overaccumulation was a special capacity in As-hyperaccumulating ferns.

Chapter 4: Effect of phosphorus accumulation states on arsenic uptake, transportation and accumulation in *Pteris vittata*

Pteris vittata can overaccumulate P in fronds as shown in Chapter 3.Many experiments are conducted of the competition of As(V) and P_i uptake in *P. vittata*. Under hydroponic conditions [4],[5]. However, the interaction between As(V) uptake and transportation with P accumulated inside the *P. vittata* remain unknown.

With the data presented in this study, the effect of P overaccumulated states on As uptake, transportation and accumulation in *P. vittata* seem to be established with some certainty: As(V) uptake via the phosphate uptake system, suppressed by the P overaccumulated states in *P. vittata*. The As(V) reductase in roots was inhibited by P overaccumulated states, and decreased the ratio of As(III) to As in the whole plants and suppressed the As(III) xylem loading. The P overaccumulated states had no significant effect on As distribution from roots to fronds (Fig. 4, 5). The present findings suggest that P overaccumulation states in *P. vittata* only affect the As metabolism in root.

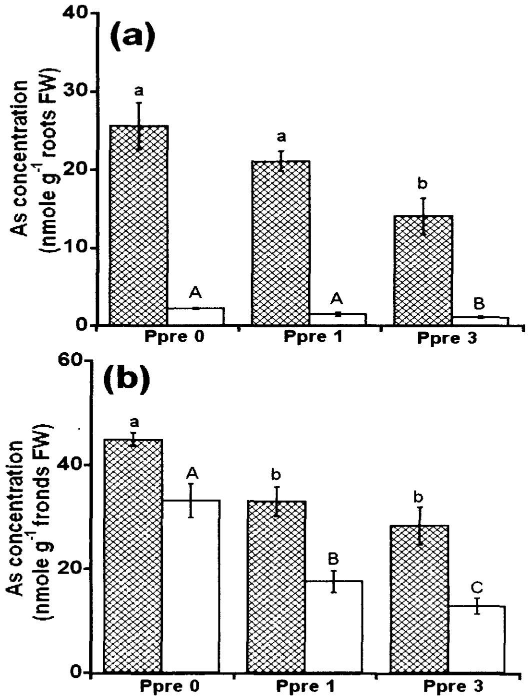

Fig. 4 Concentrations of AsT (cross-hatched bars) and As(III) (open bars) in the roots (**a**) and fronds (**b**) of *P. vittata* affected by different P accumulation states (Ppre 0, Ppre 1 and Ppre 3) in As(V) uptake experiment. AsT= As(III) + As(V). Data are means ± SE (n = 3). The different lowercase / capital letters above the cross hatched / open columns indicate significant differences ($P < 0.05$) between them.

Chapter 5: Conclusion

In this research, I found that *P. vittata* had a highly efficient As(III) xylem transport system compared with non-hyperaccumulating plants, but both the processivity of the efflux and the frond translocation were similar within 24 h, and low As(V) reduction capacity can reduce the As(III) efflux to the external medium, and a limited As(V) efflux from *P. vittata*. For the first

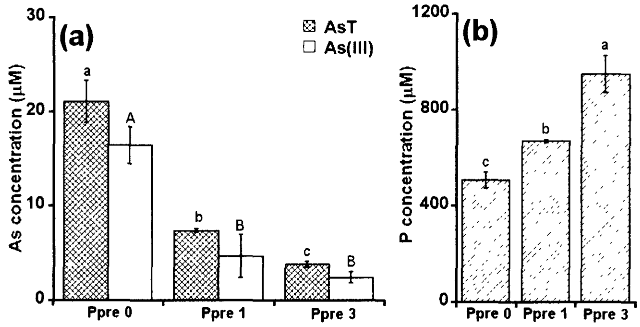

Fig. 5 Concentrations of AsT (cross-hatched bars), As(III) (open bars) (**a**) and P (hatched bars) (**b**) in the xylem sap samples collected from As(V)-treated plants at different P accumulation states(Ppre 0, Ppre 1 and Ppre 3). Data are means ± SE (n = 3). The different lowercase / capital letters above the cross hatched and hatched / open columns indicate significant differences ($P < 0.05$) between them.

time in the world, I observed that *P. vittata* can overaccumulate P in fronds like As and this special capacity only observed in As-hyperaccumulating ferns. P overaccumulation states suppressed As(V) uptake and As(III) xylem loading in *P. vittata*. The interactions of P and As in *P. vittata* need further study in molecule level.

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[2] Xu XY, McGrath SP, Zhao FJ (2007) Rapid reduction of arsenate in the medium mediated by plant roots. New Phytol 176:590–599

[3] Su YH, McGrath. SP, Zhu. YG, Zhao. FJ (2008) Highly efficient xylem transport of arsenite in the arsenic hyperaccumulator *Pteris vittata*. New Phytol 180: 434－441

[4] Tu. C, Ma L (2003) Effects of arsenate and phosphate on their accumulation by an arsenic-hyperaccumulator *Pteris vittata* L. Plant Soil 249: 373-382

[5] Tu. S, Ma LQ (2003) Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. Environ. Exp. Bot 50: 243–251

論文審査結果の要旨

汚染土壌の浄化方法のひとつとして植物を利用した浄化法（ファイトレメディエーション）がある。ヒ素汚染土壌の浄化にはヒ素超蓄積性植物であるモエジマシダ（*Pteris vittata* L.）が使用されているが、モエジマシダ体内におけるヒ素の動態は部分的にしか検討されておらず、その全体像の解明は重要な課題となっている。本論文は、モエジマシダの根におけるヒ素の取り込みと輸送、および地上部における蓄積について取りまとめたもので、以下の５章で構成されている。

第１章では、植物体内におけるヒ素の動態に関して概観するとともに、特にモエジマシダにおけるヒ素の取り込み、移動、蓄積についての現時点までの知見が整理されている。

第２章では、モエジマシダにおいて、根から取り込まれたヒ酸（As(V)）がヒ酸還元酵素（AsR）により亜ヒ酸（As(III)）に還元されるが、そのAs(III)の約半分が根から外部に排出されることが初めて示されている。また、AsR が根茎部に局在するとしたモデルを提案し、そのモデルによりモエジマシダの根部におけるヒ素の動態を表現している。さらに、根から導管への As(III)の輸送が効率的であるというこれまでの定説を否定する事実を提示している。

第３章では、ヒ素超蓄積植物であるモエジマシダがリンを過剰に蓄積する能力を有することを初めて示している。リン酸が十分に供給される土壌で栽培した場合、ヒ素の超蓄積を示さないシダと比較してモエジマシダでは 3 倍程度のリンが蓄積するが、その蓄積量が羽片の乾燥重量あたり 0.5mol/kg 以上になると生育阻害が現れることを明らかにしている。

第４章では、過剰にリンが蓄積した状態のモエジマシダにおいて、ヒ素の取り込み・輸送・蓄積にどのような影響が現れるのかを検討し、その影響は主として根からの As(V)の取り込みと根茎部での AsR の活性に現れることを明らかにしている。

第５章では本研究で得られた結果が総括されている。

以上のように、本論文では従来解明されていなかったモエジマシダの根からのヒ素の取り込みと輸送過程の詳細を実験的に明らかにし、今後の分子生物学的研究の端緒となる知見を数多く提示しており、学術的な意義は高いものがある。またリンとヒ素の吸収や排出挙動など実際のヒ素汚染土壌のファイトレメディエーションに活用できる知見も提示されており、実用上の価値も高い。

よって，本論文は博士(環境科学)の学位論文として合格と認める。